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Evaluation of Anti-diabtic and Anti-epileptic Activities of *Achillea santolina* Crude Methanolic Extract

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ABSTRACT

Achillea santolina is a traditional medicinal plant and used for various disorders in Balochistan. Current study was carried out to evaluate the antiepileptic and antidiabetic activities of *Achillea santolina* whole plant methanol extract. Preliminary phytochemical tests were carried out to determine the active constituents. Acute toxicity test was carried out to find out toxic effects of the plant. Anti-epileptic activities were carried out by Strychnine, INH and PTZ induced convulsions on mice. Antidiabetic activities were carried out by alloxan monohydrate induced diabetes. Phytochemical tests reveal the presence of carbohydrate, fixed oils, proteins, terpenoids, steroids, flavonoids and tannins. *A. santolina* crude methanolic extract did not produced any toxicity up to the dose 2g/kg oral dose. Antiepileptic activities were carried out by Strynchnine, INH and PTZ induced convulsions, *A. santolina* crude methanolic extract showed significant ($p<0.05$) effects, the onset duration of convulsions were significantly decreased. In antidiabetic activities *A. santolina* crude methanolic extract showed significantly ($p<0.05$) decreased the blood glucose level in diabetic control rabbits. It is concluded that *A. santolina* possess significant antidiabetic and antiepileptic effects.

INTRODUCTION

Epilepsy is characterized by unpredictable and recurring occurrence of a temporary behavioural disturbance, including convulsions, due to disturbed synchronous and rhythmic activation of brain neurons. It is frequent neurological disease, around 1% of the world's population is impacted by it[1].

Although the majority of epileptic patients have their seizures well controlled by anti - epileptic drugs available in the market, there still are >30% of patients who are suffering from medically convulsive status epilepticus and roughly about 40 % patients of epilepsy who are affected by a variety of adverse reactions and seizures resistance to present anti - epileptic drugs. As a result, numerous researchers work to create new methods of treating epilepsy, such as finding new antiepileptic components in herbal remedies[2]. Preparations containing herbal drugs are utilized for treatment of epilepsy are famous in numerous areas of the globe. Scientifically these herbal drugs have shown excellent anticonvulsant effects in experimental animals for anticonvulsant screening[3].

To treat hyperglycemia, a wide variety of anti-diabetic medications are currently on the market. Numerous of these synthetic medications are either driven or semi synthetically produced from plants, which are always thought of as one of the most trustworthy sources of disease-curing compounds. According to recent studies, plants and plant derivatives can have a potential anti-diabetic effect[4]. Some of the drawbacks of modern standard anti-diabetic medications include affordability, cost, accessibility, tolerance, and reduced effectiveness. Medicinal plants are commonly employed as an alternative medicine for treatment diabetes mellitus[5,6]. Especially in some countries, phytotherapy still has a substantial impact on disease prevention, especially for those with very low resources[7].

Achillea santolina (Asteraceae) is a popular remedy for gastrointestinal issues, as well as an anti- diuretic and inflammatory agent[8]. In Balochistan it is found Kalat, Mangocher, Harboi, Nichara, Surab, zairat and Hanna Urak[9]. The decoction of whole is utilized for stomach pain, diarrhea n children and fever and also used to cure jaundice[10].

Phytochemical analysis of *A. santolina* revealed the existence of alkaloid, saponins, tannins, resins, sterols, flavonoids, carbohydrates and volatile oils[11]. Previously its anti-inflammatory[11], anti-leishmanial[8], anti-diabetic[12], anti-oxidant and antimicrobial activities[13] have been reported.

Current study is carried out to evaluate the anti-epileptic and anti-diabetic potential of the *A. santolina* crude methanolic extract.

MATERIAL AND METHODS

Plant material

The Kalat district of Balochistan was used as the source for the collection of plant material. The obtained plant material was dried in shade, converted into powder, and then soaking was done methanol. The filtrate was then evaporated using a rotary evaporator under reduced pressure, yielding a light brown semi-solid residue.

Animals

Swiss albino mice 25-30 g weight and rabbits weighing about 1000–1500 g were used in experiments. They were housed in accordance with the National Institute of Health's (NIH) standards.

Phytochemical Analysis

Phytochemical analysis was carried out to confirm the existence of numerous chemical constituents by employing standard procedures[14].

Acute Toxicity

The previously mentioned procedure was used to test mice for *A. santolina* acute toxicity. Five groups of the animals were formed (n = 6). As a control, Group I was given oral administration of normal saline containing 2% gum acacia suspension (vehicle). *A. santolina* was administered orally at doses of 100, 250, 500, 750mg, 1000mg, 1500mg and 2000 mg/kg, respectively. Each animal was monitored for the following 03 days afterward administration of drug any physical, behavioural, toxic or pharmacological effects. Included parameters like writhing, hypersensitivity, respiratory changes, lacrimation, convulsions, ataxia, salivation, temperature, catalepsy and spontaneous activities were observed[15].

Antiepileptic Activity

Group of animals

All the animals were divided into four groups of 06 mice each at random. Control i.e. Group I (0.1 ml Distilled water), Group II: 2 mg/kg of diazepam. *A. santolina* methanolic extract was administered 250 mg/kg and 500 mg/kg P.O. for Groups III, IV.

Strychnine-induced seizures

Strychnine-induced seizures were used to test *A. santolina* anticonvulsant activity, and the results were compared to those of a reference standard drug (diazepam). Prior to the day of the experiment, each mouse received the appropriate dosage of extract and standard for 15 days. After receiving their respective treatments for 30 minutes on the 16th day, all animals received an intraperitoneal injection of strychnine at a dose of 1 mg/kg. They were each placed in a 20 cm by 15 cm clear cage and evaluated independently. Three different parameters, i.e. onset of convulsions and duration of convulsions were noted. Mice that continued to live 30 minutes after convulsions were regarded as safe[16].

INH induced convulsions

Four distinct groups of mice, of either sex, containing 6 mice in each were utilized. Group I received the vehicle, Phenobarbitone sodium was administered to group II at a dose of 40 mg/kg, i.p. *A. santolina* was administered to groups III and IV at doses of 250 and 500 mg/kg, p.o., respectively. INH, at a dose of 4 mg/kg was given after I hour of treatment to all groups[16].

PTZ induced convulsions

Four distinct groups mice, of either sex, containing 6 mice in each were utilized. Group I received the vehicle, Phenobarbitone sodium was administered to group II at a dose of 40 mg/kg, i.p. *A. santolina* was administered to groups III and IV at doses of 250 and 500 mg/kg, p.o. respectively. PTZ (75 mg/kg, i.p.) was given after I hour of treatment to all groups[17].

Antidiabetic activity

Diabetic rabbit preparation

A group of rabbits weighed 1000–1500 g were given an intravenous injection of 150 mg/kg of alloxan monohydrate. The blood glucose levels of all surviving rabbits were measured eight days following injection. In other experiments, diabetic rabbits with blood glucose levels between 250 and 500 mg/100 ml were employed.

The rabbits were separated into four groups of five at random. Animals in groups I were normal and healthy (non-diabetic), and alloxan (120 mg/kg, body weight) was administered to groups II–V to cause their animals to develop diabetes. Group I only received 20 ml of distilled water. Group II was served as diabetic control, Glibenclamide at 5mg/kg was administered to group III. Animals in groups IV and V received oral treatments of 250 and 500 mg/kg of *A. santolina* methanol extract, respectively. Their blood was drawn from an ear vein after administering the extract, and blood sugar levels were checked one hours later. Their blood glucose levels were compared to the negative control at days 1, 3, 6, 9, 12, and 15[18,19].

RESULTS

Phytochemical tests

The results remained positive for Carbohydrate, Fixed oils, Proteins, Terpenoids, steroids, flavonoids and tannins (Table 1).

Acute toxicity test

In acute toxicity test, *A. santolina* did not produced any mortality, maximum dose administered was dose of 2000mg/kg and there was no sign any other toxicity (table 2).

Antiepileptic activity

Strychnine induced convulsions

In this test convulsion onset was 2.02±0.03 minutes and convulsion duration was 2.17±0.04 min for group I, for group II convulsion onset was 5.24±0.08 and convulsion duration was 4.22±0.03, for group III, convulsion onset was 4.04±0.18 and convulsion duration was

2.22±0.07, for group IV, convulsion onset was 4.54±0.12 and convulsion duration was 4.54±0.12 (table 3).

INH induced convulsions

In this test convulsion onset was 2.39±0.14 and convulsion duration was 2.37±0.12 for group I, for group II convulsion onset was 7.07±0.28 and convulsion duration was 3.36±0.11, for group III, convulsion onset was 4.61±0.11 and convulsion duration was 2.49±0.17, for group IV, convulsion onset was 6.73±0.04 and convulsion duration was 3.35±0.09 (table 4).

PTZ induced convulsions

In this test convulsion onset was 2.17±0.04 and convulsion duration was 2.18±0.08 for group I, for group II convulsion onset was 6.19±0.03 and convulsion duration was 3.83±0.18, for group III, convulsion onset was 4.42±0.08 and convulsion duration was 2.13±0.07 for group IV, convulsion onset was 5.19±0.12 and convulsion duration was 2.63±0.08 (table 4).

Anti-diabetic activity

In comparison to the diabetic control, on day one, the methanolic extract of *A. santolina* significantly lowered blood glucose levels. *A. santolina* plant extract tested showed a significant reduction in blood glucose levels on the third, sixth, ninth, twelfth, and fifteenth days compared to the diabetic control rabbits; however, the decreasing effect was more pronounced in the diabetic rabbits treated with the 500mg/kg of *A. santolina* methanolic extract during this period. Similar to the pattern revealed by the methanolic plant extract, glibenclamide significantly reduced blood glucose levels.

DISCUSSION

Overall, the results of the study show that the methanol extract of *A. sanolina* had a dose-dependent protective effect on mice against strychnine, PTZ, and INH-induced convulsions. The existence of terpenoids and flavonoids in the *A. santolina* extract may be responsible for its anticonvulsant properties. Flavonoids, (such as quercetin and kampfrol), have been shown to shorten the duration of generalised seizures in the PTZ model[20,21].

In rat neural cultures, kaempferol also exhibits a moderate anticonvulsant action against PTZ-induced seizures and protective properties against NMDA-induced neurotoxicity. Due to their modulating the expression of GABAA receptors and anti-inflammatory effects in the brain,

flavonoids (a polyphenolic class to which quercetin and kaempferol belong) are thought to have antiepileptic activity[22]. It seems reasonable to assume that *A. santolina* may contain some compounds that might have anticonvulsant effect based on the findings of the current investigation, several bioactive substances, including diterpenoids, have been discovered through phytochemical research on *A. santolina*, and these substances are suggested to prevent the development of seizures and synaptic remodelling associated with seizures[21].

The crude extract of *A. santolina* demonstrated significant antidiabetic action in rabbits with alloxan-induced diabetes, providing a scientific justification for the plant's traditional applications. In this study, the entire plant extract's phytochemical examination revealed the existence of saponins, flavonoid, alkaloids and phenols, and these compounds have impact on the release of insulin by pancreatic beta-cells[23]. As a result, phytoconstituents present in the methanol extract of *A. santolina* could be the cause of its hypoglycemic effects.

Table 1. Phytochemical tests of *A. santolina* crude methanolic extract

S no.	Test	Result
1	Carbohydrate	Present
2	Fixed oils	Present
3	Proteins	Present
4	Terpenoids	Present
4	Steroids	Present
5	Glycosides	Absent
5	Cardiac glycosides	Absent
6	Anthraquinone glycosides	Absent
7	Flavonoids	Present
8	Tannins	Present

Table 2: Acute toxicity of *A. santolina* crude methanolic extract on rats

SNo	**Treatment**	**No of deaths**
1	Control	Nil
2	*A. A. santolina* 100mg/kg	Nil
3	*A. santolina* 200mg/kg	Nil
4	*A. santolina* 500mg/kg	Nil
5	*A. santolina* 750mg/kg	Nil
6	*A. santolina* 1000mg/kg	Nil
7	*A. santolina* 1500mg/kg	Null
8	*A. santolina* 2000mg/kg	Null

N=06

Table 3: Antiepileptic activity (Strychnine induced convulsions) of *A. santolina* crude methanolic extract

SNo	Treatment	Onset of Convulsion	Duration of Convulsions
1	Control	2.02±0.03	2.17±0.04
2	*Standard drug* 5mg/kg	5.24±0.08	4.22±0.03
3	*A. santolina* 250mg/kg	4.04±0.18	2.22±0.07
4	*A. santolina* 500mg/kg	4.54±0.12	3.40±0.08

Table 4: Antiepileptic activity (INH induced convulsions) of *A. santolina* crude methanolic extract

SNo	Treatment	Onset of Convulsion	Duration of Convulsions
1	Control	2.39±0.14	2.37±0.12
2	Phenobarbitone sodium 20mg/kg	7.07±0.28	3.36±0.11
3	*A. santolina* 250mg/kg	4.61±0.11	2.49±0.17
4	*A. santolina* 500mg/kg	6.73±0.04	3.35±0.09

Table 5: Antiepileptic activity (PTZ convulsions) of *A. santolina* crude methanolic extract

SNo	Treatment	Onset of Convulsion	Duration of Convulsions
1	Control	2.17±0.04	2.18±0.08
2	Phenobarbitone sodium 40mg/kg	6.19±0.03	3.83±0.18
3	*A. santolina* 250mg/kg	4.42±0.08	2.13±0.07
4	*A. santolina* 500mg/kg	5.19±0.12	2.63±0.08

Table 6: Anti- diabetic activity of *A. santolina* crude methanolic extract

SNo	Treatment	Day 1	Day 03	Day 06	Day 09	Day 12	Day 15
1	Control	111.13+0.41	12.76+0.23	113.43+0.27	114.05+0.34	113.00+0.40	113.24+0.58
	Diabetic control	311.54+0.66	326.75+0.82	333.04+0.87	330.29+1.60	321.45+0.46	312.86+0.97
2	Glibenclamide 5mg/kg	282.36+0.45	272.16+0.40	251.22+0.31	222.49+0.50	182.34+0.30	172.04+0.62
3	*A. santolina* 250mg/kg	301.59+0.26	292.26+0.64	282.02+0.32	246.65+0.93	256.35+0.62	228.26+0.50
4	*A. santolina* 500mg/kg	302.20+0.32	288.44+0.38	275+0.49	258.95+0.61	233.34+0.97	202.53+0.41

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